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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
PAK, YONG D	

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 07/27/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/031,695	HAUER ET AL.	
	Examiner	Art Unit	
	Yong D. Pak	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *03 July 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-12,14 and 16-18* is/are pending in the application.

4a) Of the above claim(s) *1-11* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *12,14 and 16-18* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

This application is a 371 of PCT/EP00/07252.

The amendment filed on July 3, 2006, amending claims 12, 14 and 17-18, has been entered.

Claims 1-12, 14 and 16-18 are pending. Claims 1-11 are withdrawn. Claims 12, 14 and 16-18 are under consideration.

Response to Arguments

Applicant's amendment and arguments filed on July 3, 2006, have been fully considered and are deemed to be persuasive to overcome the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 12 and claims 14 and 16-18 depending therefrom are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 12 recites the phrases "amino acid sequence containing SEQ ID NO:2". The metes and bounds of the phrase in the context of the claim are not clear. It is not clear to the Examiner how an amino acid sequence "contains" amino acid residues. Examiner suggests amending the phrase as "amino acid sequence comprising SEQ ID NO:2".

Claims 12 and 14 and claims 16-18 depending therefrom are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 12 recites the phrase "an amino acid sequence of SEQ ID NO:2". The metes and bounds of this phrase in the context of the above claim are not clear to the Examiner. It is not clear whether the polypeptide comprises a fragment of SEQ ID NO:2 or the full length of the amino acid sequence of SEQ ID NO:2. A perusal of the specification did not provide the Examiner with a specific definition for the above phrase. As applicants have not provided a definition for the above phrase, Examiner has interpreted the claims broadly to mean that polypeptide comprising "an amino acid of SEQ ID NO:2" encompasses fragments of SEQ ID NO:2. Examiner requests clarification of the above phrase and suggests amending the claim by replacing "an" with "the" in the above phrase.

Claim 12 and claims 16-18 depending therefrom are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 12 recites the phrase "isolating resulting hydroxylated product from the medium". This phrase is not clear to the Examiner. Modified P450 monooxygenases hydroxylate both at the terminal and subterminal positions of a carboxylic acid. Therefore, a) it is not clear to the Examiner how applicants distinguish or direct the enzyme to make only subterminally hydroxylated products and b) it is not clear to the examiner the steps of isolating subterminally hydroxylated carboxylic acids from terminally hydroxylated carboxylic acids. In the context of the above, Examiner takes the position that these claims are incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted steps are: steps in isolating subterminally hydroxylated carboxylic acids from terminally hydroxylated carboxylic acids and directing the enzyme towards making only "subterminally hydroxylated aliphatic carboxylic acids".

In response to the previous Office Action, applicants have traversed the above rejection. Applicants argue that the degree of purity of the obtained product is irrelevant since the instant invention is drawn to a method of hydroxylating aliphatic C8-C12-carboxylic acids. Examiner respectfully disagrees. The rejection does not question the degree of purity of the products but how subterminally hydroxylated carboxylic acids are separated from terminally hydroxylated carboxylic acids or how the enzymes are directed towards making only subterminally hydroxylated aliphatic carboxylic acids.

Applicants also argue that a skilled person may rely on routine methods in order to separate the obtained reaction mixtures. Examiner respectfully disagrees. The claims do not recite a step of actually isolating "subterminally hydroxylated" products. The claims only recite a step of isolated "hydroxylated products", which includes both subterminally and terminally hydroxylated products. Therefore, it is not clear if subterminally hydroxylated carboxylic acids are separated from terminally hydroxylated carboxylic acids of if the enzymes are directed towards making only subterminally hydroxylated aliphatic carboxylic acids.

Hence the rejection is maintained.

Claim 12 and claims 14 and 16-18 depending therefrom are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 12 recites the phrase "is derived from *Bacillus megaterium*". The metes and bounds of this phrase are not clear to the Examiner. Literally, while the term "derived" means "to isolate from or obtain from a source", the above term could also mean "to arrive at by reasoning i.e., to deduce or infer" or also as "to produce or obtain from another substance". Therefore, it is not clear to the Examiner either from the specification or form the claims as to what applicants mean by the above phrase. It is not clear to the Examiner whether the monooxygnease "derived from *Bacillus megaterium* encompasses a single specific enzyme (SEQ ID NO:2), as in isolated from *Bacillus megaterium,* or whether it encompasses recombinants, variants and mutants of

the monooxygenase of SQ ID NO:2 or modified monooxygenase from any other source and labeled as a monooxygenase "derived from *Bacillus megaterium*". As applicants have not provided a definition for the above phrase, Examiner has interpreted the claims broadly to mean that a monooxygenase "derived from *Bacillus megaterium*" encompasses polypeptides which are recombinants, variants or mutants of any monooxygenase. Examiner has given the same interpretation while considering the claims for all other rejections. The rejection can be overcome by amending the phrase to recite "wherein the cytochrome P450 monooxygenase is isolated from *Bacillus megaterium*".

In response to the previous Office Action, applicants have traversed the above rejection. Applicants argue that the claim is definite because one having ordinary skill in the art would readily understand the meaning of the term "derived" in the context of the instant application and because claims are read in light of the specification. Examiner respectfully disagrees. MPEP 2111 states that "claims must be given their broadest reasonable interpretation consistent with the specification." As applicants have not provided a definition for the above phrase, Examiner has interpreted the claims broadly to mean that a monooxygenase "derived from *Bacillus megaterium*" encompasses polypeptides which are recombinants, variants or mutants of any monooxygenase.

Applicants also argue that the claim is definite because the specification on pages 2-3 explains "what is done when a monooxygenase is derived from *B. megaterium*". Examiner respectfully disagrees. On pages 2-3 of the specification does not provide a clear definition of the above phrase; whether the monooxygnease "derived

from *Bacillus megaterium"* encompasses a single specific enzyme (SEQ ID NO:2), as in isolated from *Bacillus megaterium,* or whether it encompasses recombinants, variants and mutants of the monooxygenase of SQ ID NO:2 or modified monooxygenase from any other source and labeled as a monooxygenase "derived from *Bacillus megaterium".*

Hence the rejection is maintained.

Claim 14 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 14 recites groups of amino acid substitutions, such as in claim 14 part f) "F87A L188K A74G and R47F". It is not clear if the amino acid substitutions in these groups is in the alternative or is all-inclusive.

Applicants argue that the claims have been amended to alleviate the rejection. Examiner respectfully disagrees. For example in part f), there is a conjunction missing between "F87A", L188K" and "A74G". Therefore, it is not clear if the amino acid substitutions in part d)-i) is in the alternative or is all-inclusive. Hence the rejection is maintained.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 12, 14 and 16-18 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 12, 14 and 16-18 are drawn to a method for the enzymatic production of subterminally hydroxylated aliphatic carboxylic acids with a cytochrome P450 monooxygenase derived from a *Bacillus megaterium* cytochrome P450 monooxygenase BM-3 having the amino acid sequence of SEQ ID NO:2 comprising at least one mutation recited in the claims or any other amino acid modification and having an altered activity or regioselectivity. Further, the claims are not limited to variants of SEQ ID NO:2 consisting of the recited mutations since the claims recite that the P450 monooxygenases are derived from SEQ ID NO:2, which includes any or all mutant, variants and recombinants thereof, and the limitation of comprising substitutions at the recited positions provides no description on the structure of other parts of the mutant monooxygenase. While the polypeptide used in the method can comprise the recited substituted amino acids, the same polypeptide can comprise any number of amino acids in other positions. Thus, the claims encompass a method for the production of subterminally hydroxylated aliphatic carboxylic acids using any recombinants, variants and mutants of cytochrome P450 monooxygenase derived from a *Bacillus megaterium* cytochrome P450 monooxygenase, including any or all mutants, variants and recombinants thereof. Therefore, the claim is drawn to a method of using a genus of polypeptides having any structure. The specification only teaches a method for

hydroxylating 15-para-nitrophenoxycarboxylic acids (pNCA), 12-pNCA, 10-pNCA or 8-pNCA with a modified cytochrome P450 monooxygenase of SEQ ID NO:2 having mutations at residue 26, 47, 74, 87, 188 or 354 of SEQ ID NO:2 expressed in a host cell comprising a polynucleotide encoding said modified monooxygenases. These limited examples are not enough and does not constitute a representative number of species to describe the whole genus and there is no evidence on the record of the relationship between the structure of a modified cytochrome P450 monooxygenase of SEQ ID NO:2 consisting of substitutions at residues 26, 47, 74, 87, 188 or 354 of SEQ ID NO:2 and the structure of any recombinants, variants and mutants of any cytochrome P450 monooxygenase derived from SEQ ID NO:2. Therefore, the specification fails to describe a representative species of the genus comprising variants and mutants of any recombinants, variants and mutants of any cytochrome P450 monooxygenase derived from SEQ ID NO:2 use to produce subterminally hydroxylated aliphatic carboxylic acids.

Given this lack of description of the representative species encompassed by the genus of the claims, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the inventions of claims 12, 14 and 16-18.

Applicant is referred to the revised guidelines concerning compliance with the written description requirement of U.S.C. 112, first paragraph, published in the Official Gazette and also available at www.uspto.gov.

In response to the previous Office Action, applicants have traversed the above rejection. Applicants argue that a single species can be enough to support a genus

when it conveys to the ordinarily skilled artisan the necessary common attributes possessed by the genus and since the instant specification provides a "representative number" of species, mutants of SEQ ID NO:2, the claims are fully described by the specification. Examiner respectfully disagrees. The claims are not only drawn to mutants of SEQ ID NO:2 consisting of the recited substitutions, but any mutants of any monooxygenase "derived" from SEQ ID NO:2, including any or all mutants, variants and recombinants thereof, comprising the recited substitutions. The genus comprising any or all recombinants, variants and mutants of any monooxygenase does not possess any common attributes other than having monooxygenase activity. Therefore, the specification lacks description of a representative number of species to describe the whole genus. As discussed in the written description guidelines, the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. A representative number of species means that the species which are adequately described are representative of the entire genus. **Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus.** Satisfactory disclosure of a representative number depends on whether one of skill in the art would recognize that

the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed. For inventions in an unpredictable art, adequate written description of a genus which embraces widely variant species cannot be achieved by disclosing only one species within the genus. In the instant case the claimed genus used in the method includes species which are widely variant in structure. As such, the disclosure solely functional features present in all members of the genus is sufficient to be representative of the attributes and features of the entire genus.

Hence the rejection is maintained.

Claims 12, 14 and 16-18 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of enzymatic production of specific subterminally hydroxylated aliphatic carboxylic acids by using a modified cytochrome P450 monooxygenase with SEQ ID NO:2 consisting of single or multiple mutations at residue 26, 47, 74, 87, 188 or 354 of SEQ ID NO:2 with 15-para-nitrophenoxycarboxylic acids (pNCA), 12-pNCA, 10-pNCA or 8-pNCA as substrates, does not reasonably provide enablement for a method for the production of subterminally hydroxylated aliphatic carboxylic acids wherein said method encompasses the use of any cytochrome P450 monooxygenase derived from SEQ ID NO:2. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate

in scope with these claims. (See rejection of "derived" under 35 U.S.C. 112, 2nd paragraph).

Factors to be considered in determining whether undue experimentation is required, are summarized in In re Wands (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)) as follows: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claim(s).

Claims 12, 14 and 16-18 are drawn to a method for the enzymatic production of subterminally hydroxylated aliphatic carboxylic acids with a cytochrome P450 monooxygenase derived from a *Bacillus megaterium* cytochrome P450 monooxygenase BM-3 having the amino acid sequence of SEQ ID NO:2 comprising at least one mutation recited in the claims or any other amino acid modification and having an altered activity or regioselectivity. Further, the claims are not limited to variants of SEQ ID NO:2 consisting of the recited mutations since the claims recite that the P450 monooxygenases are derived from SEQ ID NO:2, which includes any or all mutant, variants and recombinants thereof, and the limitation of comprising substitutions at the recited positions provides no description on the structure of other parts of the mutant monooxygenase. While the polypeptide used in the method can comprise the recited substituted amino acids, the same polypeptide can comprise any number of amino acids in other positions. Thus, the claims encompass a method for the production of

any or all subterminally hydroxylated aliphatic carboxylic acids using any recombinants, variants and mutants of cytochrome P450 monooxygenase derived from a *Bacillus megaterium* cytochrome P450 monooxygenase, including any or all mutants, variants and recombinants thereof. Thus, the claims encompass a method for the production of subterminally hydroxylated aliphatic carboxylic acids using any recombinants, variants and mutants of any P450 monooxygenase derived from SEQ ID NO:2. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of P450 monooxygenase variants and mutants, broadly encompassed by the claims. The claims encompass compounds with widely varying structure and properties. However, in this case the disclosure is limited to a method for hydroxylating 15-para-nitrophenoxycarboxylic acids (pNCA), 12-pNCA, 10-pNCA or 8-pNCA with a modified cytochrome P450 monooxygenase of SEQ ID NO:2 having mutations at residue 26, 47, 74, 87, 188 or 354 of SEQ ID NO:2 expressed in a host cell comprising a polynucleotide encoding said modified monooxygenases. It would require undue experimentation of the skilled artisan to hydroxylate any carboxylic acids.

Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function.

However, in this case the disclosure is limited to a method for hydroxylating 15-para-nitrophenoxycarboxylic acids (pNCA), 12-pNCA, 10-pNCA or 8-pNCA with a modified cytochrome P450 monooxygenase of SEQ ID NO:2 having mutations at residue 26, 47, 74, 87, 188 or 354 of SEQ ID NO:2 expressed in a host cell comprising a polynucleotide encoding said modified monooxygenases. It would require undue experimentation of the skilled artisan to make and use the claimed variants and mutants of any P450 monooxygenases. In view of the great breadth of the claim, amount of experimentation required to make the claimed polynucleotides, the lack of guidance, working examples, and unpredictability of the art in predicting function from a polypeptide primary structure, the claimed invention would require undue experimentation. As such, the specification fails to teach one of ordinary skill how to use the full scope of the polypeptides encompassed by this claim.

While enzyme isolation techniques, recombinant and mutagenesis techniques are known, and it is routine in the art to screen for multiple substitutions or multiple modifications as encompassed by the instant claims, the specific amino acid positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of the claims which encompass a method for the enzymatic production of subterminally hydroxylating

aliphatic carboxylic acids using any or all mutants and variants of any P450 monooxygenase, because the specification does not establish: (A) regions of the substrate binding region of any P450 monooxygenase which may be modified without affecting P450 monooxygenase activity or having an altered substrate profile; (B) the general tolerance of P450 monooxygenase to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any amino acid residue with an expectation of obtaining the desired biological function; (D) aliphatic carboxylic acids which are subterminally hydroxylated with any P450 monooxygenases; (E) a rational and predictable scheme for selecting aliphatic carboxylic acids with an expectation of obtaining a subterminally hydroxylated aliphatic carboxylic acids by incubating said substrates with any P450 monooxygenase; and (F) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including a method for the production of subterminally hydroxylated aliphatic carboxylic acids using any or all variants and mutants of any P450 monooxygenase. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of mutants and variants of any P450 monooxygenase having the desired biological characteristics recited in the claim is unpredictable and the experimentation left to those skilled in the art is unnecessarily,

and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

In response to the previous Office Action, applicants have traversed the above rejection.

Applicants argue that the Examiner has recited the standard USPTO phraseology in rejection the instant claims, alleging that the instant disclosure presents no guidance or working examples of the use of any or all mutants, recombinants, or variants of any or all cytochrome P45 monooxygenase, when the claims are drawn to a method of using "a nucleic acid sequence encoding a monooxygenase which is derived from *Bacillus megaterium* cytochrome P450 monooxygenase BM-3 with an amino acid sequence of SEQ ID NO:22 which has a function mutation in the amino acid sequence region 86-88". As stated in the above rejection, the claims are not limited to variants of SEQ ID NO:2 consisting of the recited mutations since the claims recite that the P450 monooxygenases are "derived" from SEQ ID NO:2, which includes any or all mutant, variants and recombinants thereof, and the limitation of comprising substitutions at the recited positions provides no description on the structure of other parts of the mutant monooxygenase. While the polypeptide used in the method can comprise the recited substituted amino acids, the same polypeptide can comprise any number of amino acids in other positions. Thus, the claims encompass a method for the production of any or all subterminally hydroxylated aliphatic carboxylic acids using any recombinants, variants and mutants of cytochrome P450 monooxygenase derived from a *Bacillus*

megaterium cytochrome P450 monooxygenase, including any or all mutants, variants and recombinants thereof.

Applicants also argue that the claims are fully enabled because claims 14 and 18 indicate specific individual amino acid mutations. Examiner respectfully disagrees. The claims are not limited to variants of SEQ ID NO:2 consisting of the recited mutations since the claims recite that the P450 monooxygenases are "derived" from SEQ ID NO:2, which includes any or all mutant, variants and recombinants thereof, and the limitation of comprising substitutions at the recited positions provides no description on the structure of other parts of the mutant monooxygenase.

Applicants also argue that the rejection does not explain "why" a skilled artisan would expect any tolerance to diminish upon multiple modification. Since the claims are drawn to a P450 monooxygenase comprising any number of mutations, upon such multiple modifications (no upper limit on the number of mutations), a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. As discussed above, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a specific knowledge of and guidance with regard to which specific amino acids in the protein's sequence, can be modified such that the modified polypeptide continues to have said claimed activity. It is this specific guidance that applicants do not provide. Without specific guidance, those skilled in the art will be subjected to undue experimentation of making and testing each of the enormously large number of mutants that results from such experimentation. While the art may teach in

general the structure of P450 monooxygenase, conserved amino acid sequences, and etc, such teachings will not reduce the burden of undue experimentation on those of ordinary skill in the art.

Applicants also argue that Examiner has provided no evidence to support the assertions of undue experimentation. MPEP 2164.04 states that references "are not always required", but only specific technical reasons, which Examiner has done. As discussed in the rejection, it would require undue experimentation to practice the claimed invention because the claims are drawn to a method using any or all P450 mutants comprising the recited amino acid substitutions and any other mutations. Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. As discussed above, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a specific knowledge of and guidance with regard to which specific amino acids in the protein's sequence, can be modified such that the modified polypeptide continues to have said claimed activity. It is this specific guidance that applicants do not provide. Without specific guidance, those skilled in the art will be subjected to undue experimentation of making and testing each of the enormously large number of mutants that results from such experimentation. While the art may teach in

general the structure of P450 monooxygenase, conserved amino acid sequences, and etc, such teachings will not reduce the burden of undue experimentation on those of ordinary skill in the art.

Hence the rejection is maintained.

Claim Rejections - 35 USC § 102

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 12, 14 and 16-18 are rejected under 35 U.S.C. 102(b) as being anticipated by Graham-Lorence et al. (form PTO-1449)

Claims 12, 14 and 16-18 are drawn to a method of producing subterminally hydroxylated aliphatic carboxylic acid by reacting C_8-C_{12}-carboxylic acid derivatives with a modified P450 monooxygenase wherein said modification is a mutation at residue 87 inSEQ ID NO:2 and wherein said modified enzymes shows an altered substrate profile. (See rejection of "derivatives" under 35 U.S.C. 112, 2nd paragraph).

Graham-Lorence et al. discloses a method for producing subterminally hydroxylated aliphatic carboxylic acids with a modified P450 monooxygenase having a valine residue at position 87 of SEQ ID NO:2, wherein the mutant enzyme shows an altered substrate profile (abstract and page 1129). The method of Graham-Lorence et al. uses the reductant recited in claim 17 (page 1127). Therefore, the teachings of Graham-Lorence et al. anticipate claims 12, 14 and 16-18.

In response to the previous Office Action, applicants have traversed the above rejection. Applicants argue that Graham-Lorence et al. does not anticipate the claimed

invention because arachidonic acid is not a derivative of a C_8-C_{12}-carboxylic acid as defined in the claim and claims should not be read in a vacuum. Examiner respectfully disagrees. Because the phrase a derivative of C_8-C_{12}-carboxylic acid "selected from an alkyl ester, an amide or an anhydride thereof" is not clear and since the specification does not exclude a 20 carbon fatty acid from "derivatives of C_8-C_{12}-carboxylic acids", an arachidonic acid has been interpreted as a derivative of a C_8-C_{12}-carboxylic acid. Further, absent of any scientific or objective evidence illustrating that arachidonic acid is not a derivative of C_8-C_{12}-carboxylic acid, the reference of Graham-Lorence et al. anticipates the claimed invention.

Hence the rejection is maintained.

None of the claims are allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Yong Pak whose telephone number is 571-272-0935. The examiner can normally be reached 6:30 A.M. to 5:00 P.M. Monday through Thursday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy can be reached on 571-272-0928. The fax phone numbers for the organization where this application or proceeding is assigned are 571-273-8300 for regular communications and 703-872-9307 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 571-272-1600.

Yong D. Pak
Patent Examiner 1652

Tekchand Saidha
Primary Patent Examiner 1652